Anal. Calcd for $C_6H_{14}O_7S_2$: C, 27.48; H, 5.38; S, 24.45. Found: C, 27.64; H, 5.41; S, 24.49.

Diethyl 2-*O*-(2-Tetrahydropyranyl)-L-maleate.—To a mixture of diethyl maleate (95 g) and purified dihydropyran (46 g), concentrated HCl (0.2 ml) was added. The reaction mixture was allowed to warm and kept for 28 hr at room temperature. Neutralization of the HCl with excess of Ag_2O[6] and distillation *in vacuo*, leaving some undistilled material in the distillation flask in order to avoid any risk caused by peroxide formation, yielded 49 g of the ester, bp 118.5–119° (0.4 mm), $[\alpha]^{20}D$ −59° (*c* 6, acetone).

Anal. Calcd for $C_{13}H_{22}O_6$: C, 56.92; H, 8.08. Found: C, 56.64; H, 8.14.

2-*O*-(2-Tetrahydropyranyl)-(*S*)-1,2,4-butanetriol.—A suspension of $LiAlH_4$ (20 g) in diethyl ether (500 ml) was refluxed for 1 hr. A solution of diethyl 2-*O*-(2-tetrahydropyranyl)-L-maleate (46 g) in diethyl ether (50 ml) was added dropwise with stirring, the heat of reaction causing a gentle refluxing. After additional heating for 2 hr ethyl acetate (70 ml) was carefully added, and the reaction mixture was cooled. After successive cautious additions of water (20 ml) and 4 *N* NaOH (20 ml), the inorganic precipitate was removed by filtration, washed several times with diethyl ether (500 ml), and extracted with hot chloroform (200 ml). The combined organic solutions were dried ($MgSO_4$) and evaporated *in vacuo*. Distillation of the residue yielded 13.9 g, bp 115–123° (0.2 mm), $[\alpha]^{20}D$ −47.1° (*c* 6, acetone).

Anal. Calcd for $C_9H_{18}O_4$: C, 56.82; H, 9.54. Found: C, 57.16; H, 9.42.

(*S*)-1,2,4-Butanetriol 1,4-Bismethanesulfonate.—To a solution of 2-*O*-(2-tetrahydropyranyl)-(*S*)-1,2,4-butanetriol (5.2 g) in pyridine (15 ml), methanesulfonyl chloride (6 ml) was added dropwise while stirring at −20 to −15° over a period of 30 min, and the reaction mixture was then kept at −15 to −5° for an additional 40 min. After standing for 20 hr at about 5° the mixture was poured into ice–water (300 ml). The separated heavy oil was washed with water by repeated decantation, and dissolved in chloroform. After drying ($MgSO_4$) and evaporation *in vacuo*, the resulting crude 2-*O*-(2-tetrahydropyranyl)-(*S*)-1,2,4-butanetriol 1,4-bismethanesulfonate was refluxed in ethanol (35 ml) for 20 min after addition of methanesulfonic acid (0.3 ml). After standing at about −5° for 20 hr (*S*)-1,2,4-butanetriol 1,4-bismethanesulfonate (2.2 g) separated, mp 68–70°. Additional material (1.6 g) with the same melting point could be isolated from the mother liquor. Recrystallization from ethanol raised the melting point to 68.5–70°, $[\alpha]^{20}D$ −16.5° (*c* 6, acetone).

Anal. Calcd for $C_6H_{14}S_2O_7$: C, 27.48; H, 5.38; S, 24.45. Found: C, 27.41; H, 5.35; S, 24.36.

Acknowledgment.—The authors are indebted to the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda 14, Md., for the screening of the compounds and for making the results available.

(6) Work-up by treating with $NaHCO_3$ solution resulted in a product with decreased optical rotation.

v-Triazolo[4,5-*d*]pyrimidines. III. N-(3-Alkyl-5-amino-3H-*v*-triazolo[4,5-*d*]pyrimidin-7-yl)-amino Acids[1]

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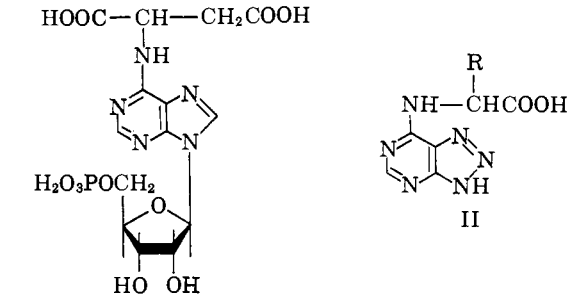
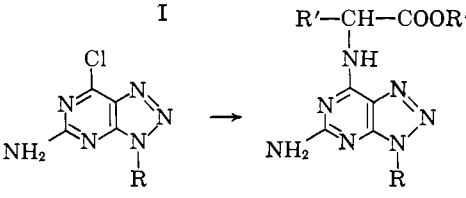
N-[9-(β-D-Ribofuranosyl)purin-6-yl]aspartic acid 5′-phosphate (I) is an intermediate in the biochemical interconversion of inosinic and adenylic acids and is, therefore, an intermediate in the biosynthesis of nucleic acids.[2] A number of other N-(purin-6-yl)amino acids have been prepared as analogs of the aspartic acid derivative.[3] In addition, Ballweg[4] has synthesized a few N-(*v*-triazolo[4,5-*d*]pyrimidin-7-yl)amino acids (II). We report here the synthesis and antitumor evaluation of several N-(5-amino-*v*-triazolo[4,5-*d*]pyrimidin-7-yl)amino acids that have an alkyl substituent at the position corresponding to that occupied by the ribofuranosyl group in I.



I

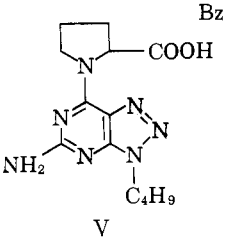
II



III, R = C_2H_5
IV, R = C_4H_9

VI, R = C_2H_5; R′ = R″ = H
VII, R = C_4H_9; R′ = $C_6H_5CH_2$; R″ = H
VIII, R = C_4H_9; R′ = $BzOOCCH_2$; R″ = Bz
IX, R = C_4H_9; R′ = $HOOCCH_2$; R″ = H
X, R = C_2H_5; R′ = $BzOOCCH_2$; R″ = Bz
XI, R = C_2H_5; R′ = $HOOCCH_2$; R″ = H

Bz = benzyl



V

The *v*-triazolo[4,5-*d*]pyrimidin-7-ylamino acids were prepared from 5-amino-7-chloro-3-ethyl-3H-*v*-triazolo-[4,5-*d*]pyrimidine[5] (III) or the 3-butyl derivative (IV). Equivalent amounts of free amino acid and triethylamine in anhydrous alcohol were employed for the preparation of the monobasic amino acid derivatives. The aspartic acid derivatives (IX and XI) were obtained by displacing the 7-chloro group of III and IV with dibenzyl aspartate and hydrogenating the resulting ester derivatives (VIII and X).

(1) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740, and by the C. F. Kettering Foundation.

(2) C. E. Carter and L. H. Cohen, *J. Am. Chem. Soc.*, **77**, 499 (1955); R. Abrams and M. Bentley, *ibid.*, **77**, 4179 (1955); I. Lieberman, *ibid.*, **78**, 251 (1956); J. M. Buchanan and S. C. Hartman, *Advan. Enzymol.*, **21**, 199 (1959).

(3) See, for example, H. Lettré and H. Ballweg, *Ann.*, **633**, 171 (1960); **656**, 158 (1962); A. Ballio and V. DiVittorio, *Gazz. Chim. Ital.*, **90**, 501 (1960); D. N. Ward, J. Wade, E. F. Walborg, Jr., and T. S. Osdene, *J. Org. Chem.*, **26**, 5000 (1961).

(4) H. Ballweg, *Ann.*, **657**, 141 (1962).

(5) Y. F. Shealy, R. F. Struck, J. D. Clayton, and J. A. Montgomery, *J. Org. Chem.*, **26**, 4433 (1961).

TABLE I
TESTS AGAINST TRANSPLANTABLE MOUSE TUMORS[a]

Compd	Tumor	Dose, mg/kg/day	Mortality	Av wt change, T/C[b]	Tumor data T/C[c]	Tumor data T/C ratio, %
V	S180	500	0/6	−0.4/−0.1	844/613	137
	Ca755	500	1/10	+3.0/+3.3	643/1062	60
	L1210	500	0/6	+2.6/+3.6	6.8/8.1	83[d]
		250	0/6	+3.3/+1.7	7.3/8.5	86
VI	S180	500	0/6	−0.5/+1.0	1202/1307	91
	Ca755	500	1/10	+1.0/+0.3	555/1025	54
VII	S180	500	0/6	−2.6/−1.0	887/942	94
	Ca755	400	3/10	+0.9/+2.2	834/1314	63
	L1210	400	0/6	+1.2/+0.7	7.5/8.5	88
VIII	S180	500	1/6	−1.7/−0.1	677/829	81
	Ca755	400	0/10	+3.0/+2.7	1535/1366	112
	L1210	400	0/6	−0.3/+0.6	8.0/9.1	88
IX	S180	500	0/6	+1.6/+3.2	1540/1720	89
	Ca755	500	3/10	+3.8/+3.1	1654/1436	115
	L1210	500	0/6	−0.6/+0.1	7.8/9.1	86
X	S180	250	0/6	−0.9/−0.5	948/1173	80
XI	S180	500	0/6	+0.2/+0.6	1131/1365	82
	Ca755	500	1/10	+3.2/+3.1	1697/1436	118
	L1210	500	0/6	−2.4/−2.3	10.2/9.8	104

[a] T = treated animals, C = control animals. [b] Average weight change of host animals in grams during the duration of the S180 and Ca755 tests and during the first 5 days of the L1210 tests. [c] Average tumor weights in milligrams for S180 and Ca755; average survival time in days for L1210. [d] Toxic.

The five amino acid derivatives and one of the ester derivatives (IX) were tested[6] against Sarcoma 180 and Adenocarcinoma 755 in mice at the highest dose levels (500 and 400 mg/kg per day) generally administered in the testing program of the Cancer Chemotherapy National Service Center.[7] Some of these compounds were also tested against mouse leukemia L1210. As shown in Table I, no significant antitumor activity was found in any of these tests except for borderline activity by the glycine derivative (VI) against Ca755 in a single, unconfirmed test.

Experimental Section

Ultraviolet spectra were recorded with a Cary Model 14 recording spectrophotometer. Solutions for ultraviolet spectral determinations were prepared by dissolving a weighed sample in 50 ml of water or ethanol and diluting 5-ml aliquot portions to 50 ml with 0.1 *N* HCl, phosphate buffer (pH 7), 0.1 *N* NaOH, or ethanol. Unless stated otherwise, melting points were determined on a Kofler Heizbank melting point apparatus and are corrected; "cap." means that the melting temperature was determined in a capillary tube.

N-(5-Amino-3-butyl-3H-*v*-triazolo[4.5-*d*]pyrimidin-7-yl)proline (V).—A mixture (protected from atmospheric moisture by a tube of $CaSO_4$) consisting of 6.00 g (27 mmoles) of IV, 3.06 g (27 mmoles) of L-proline, 2.64 g (27 mmoles) of triethylamine, and 1 l. of absolute ethanol was heated at the reflux temperature for 3 hr. The residue remaining after removal of the volatile material *in vacuo* was washed thoroughly with water and then recrystallized from 2:1 water–ethanol. The white product, isolated in two crops, amounted to 5.4 g (66%), mp 195–196°. The analytical sample, prepared by recrystallizing a crude specimen three times from water–ethanol, had the same melting point; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 263 (18.2) in 0.1 *N* HCl; 235 (17.4), 294 (15.2) in phosphate buffer (pH 7); 235 (17.3), 294 (15.0) in 0.1 *N* NaOH; 234 (19.8), 270 (sh), 291 (13.3) in ethanol.

Anal. Calcd for $C_{13}H_{19}N_7O_2$: C, 51.13; H, 6.27; N, 32.11. Found: C, 51.05; H, 6.18; N, 31.77.

N-(5-Amino-3-ethyl-3H-*v*-triazolo[4.5-*d*]pyrimidin-7-yl)glycine (VI) precipitated from a similar reaction mixture of 6.0 g of III and glycine that had been heated at the reflux temperature for 26 hr and then chilled. A mixture of the precipitate (4.2 g, mp 255–260° dec) with a second crop (980 mg) from the filtrate was dissolved in 1 *N* aqueous NaOH, treated with activated carbon, and reprecipitated by acidifying the resulting filtrate to pH 3 with HCl. Repetition of this reprecipitation procedure afforded 3.45 g (48%) of white solid: mp 271–273° dec (cap.); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 256 (15.2), 273 (12.6) in 0.1 *N* HCl; 260 (sh), 289 (12.6) in phosphate buffer (pH 7) or 0.1 *N* NaOH.

Anal. Calcd for $C_8H_{11}N_7O_2$: C, 40.50; H, 4.67; N, 41.33. Found: C, 40.52; H, 4.83; N, 41.10.

DL-N-(5-Amino-3-butyl-3H-*v*-triazolo[4.5-*d*]pyrimidin-7-yl)-phenylalanine (VII) was prepared by the procedure used to prepare V except that the reaction time was 44 hr. Addition of hot 2:1 ethanol–water to the residue remaining after the removal of volatile material from the reaction mixture gave VII as a crystalline solid in 41% yield. Recrystallization of a specimen from 50% aqueous ethanol gave the analytical sample: mp 215–217° (cap.); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 258 (16.4), 270–275 (sh) in 0.1 *N* HCl; 230–235 (sh), 265 (sh), 292 (13.0) in phosphate buffer (pH 7) and in 0.1 *N* NaOH.

Anal. Calcd for $C_{17}H_{21}N_7O_2$: C, 57.45; H, 5.96; N, 27.58. Found: C, 57.19; H, 6.27; N, 27.63.

Dibenzyl N-(5-Amino-3-butyl-3H-*v*-triazolo[4.5-*d*]pyrimidin-7-yl)aspartate (VIII).—A solution of 12.2 g (34 mmoles) of IV, 34.0 g (108 mmoles) of dibenzyl L-aspartate,[8] and 1 l. of absolute ethanol was heated at the reflux temperature for 3 hr in the absence of moisture. The oil remaining after evaporation of the the ethanol *in vacuo* was dissolved in hot benzene. Cyclohexane was added to the cloud point, the mixture was cooled slowly to room temperature and refrigerated, and the resulting yellow precipitate was removed by filtration and stirred in 50 ml of water for 10 min. The white solid that remained after the insoluble material had been separated by filtration, washed with water, and dried weighed 10 g (37%), mp 82–83°. Two additional crops of VIII amounting to 5.75 g (mp 84–85°) and 2.4 g (mp 77–79°) were obtained by concentrating the benzene–cyclohexane filtrate *in vacuo*, slurrying the residual oil with water, and recrystallizing the insoluble residue from benzene–cyclohexane. The analytical sample was prepared by recrystallizing a crude specimen from benzene–cyclohexane; mp 84–85°; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 227 (22.0), 260 (7.4), 290 (11.8) in ethanol.

Anal. Calcd for $C_{36}H_{39}N_7O_4$: C, 62.01; H, 5.81; N, 19.47. Found: C, 62.12; H, 5.83; N, 19.28.

Dibenzyl N-(5-amino-3-ethyl-3H-*v*-triazolo[4.5-*d*]pyrimidin-7-yl)aspartate (X) was obtained from III by the procedure used to

(6) Biological testing was performed by the Chemotherapy Department of Southern Research Institute under the auspices of the Cancer Chemotherapy National Service Center and under the supervision of Drs. F. M. Schabel, Jr., and W. R. Laster, Jr.

(7) *Cancer Chemotherapy Rept.*, **No. 25**, 1 (1962); **1**, 42 (1959).

(8) L. Velluz, G. Amiard, J. Bartos, B. Goffinet, and R. Heymés, *Bull. Soc. Chim. France*, 1464 (1956).

prepare VIII except that (1) the reaction time was 4 hr and (2) the benzene solution of the reaction-mixture residue was washed with water, dried ($MgSO_4$), and treated with activated carbon before the dilution with cyclohexane. The analytical sample (from benzene–cyclohexane) melted at 55°; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 227 (22.1), 260 (7.3), 289 (11.8) in ethanol.

Anal. Calcd for $C_{24}H_{25}N_7O_4$: C, 60.62; H, 5.31; N, 20.62. Found: C, 60.53; H, 5.29; N, 20.86.

N-(5-Amino-3-ethyl-3H-*v*-triazolo[4,5-*d*]pyrimidin-7-yl)aspartic Acid (XI).—A mixture of 10.1 g of X, 1 l. of absolute ethanol, and 2.0 g of 5% palladium–charcoal catalyst was treated with hydrogen at atmospheric pressure and room temperature until the calculated quantity of hydrogen had been absorbed. After the catalyst had been removed by filtration and the ethanolic filtrate concentrated to about 150 ml and chilled, a white solid precipitated; 5.16 g, mp 227–230° dec. A second portion of 540 mg (mp 225–235° dec) was obtained by evaporating the ethanol from the filtrate, dissolving the white residue in 1 *N* NaOH, and precipitating the product by acidification. A solution of the combined portions in 100 ml of 1 *N* NaOH was acidified to pH 2 with 6 *N* HCl and refrigerated. The white crystalline XI, which was separated by filtration, washed with water, and dried *in vacuo*, weighed 4.96 g (78%); mp 234–236° dec (cap.); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 256 (16.0), 273 (13.3) in 0.1 *N* HCl; 230 (16.6 sh), 265 (sh), 291 (12.9) in phosphate buffer (pH 7); 230 (16.0 sh), 265 (sh), 291 (12.8) in 0.1 *N* NaOH.

Anal. Calcd for $C_{10}H_{13}N_7O_4$: C, 40.64; H, 4.44; N, 33.21. Found: C, 40.71; H, 4.66; N, 33.14.

N-(5-Amino-3-butyl-3H-*v*-triazolo[4,5-*d*]pyrimidin-7-yl)aspartic acid (IX) was obtained by hydrogenation of VIII by the procedure for XI. The white solid remaining after removal of the catalyst and evaporation of the ethanol was recrystallized twice from 50% aqueous ethanol; yield of IX, 33%; mp 175–180° (cap.); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 256 (16.3), 274 (13.4) in 0.1 *N* HCl; 218 (18.0), 230 (sh), 265 (sh), 290 (13.1) in phosphate buffer; 230 (sh), 265 (sh), 290 (13.3) in 0.1 *N* NaOH.

Anal. Calcd for $C_{12}H_{17}N_7O_4$: C, 44.57; H, 5.36; N, 30.33. Found: C, 44.37; H, 5.36; N, 30.09.

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6-Azauracil Derivatives of Fluoropyruvic Acid[1]

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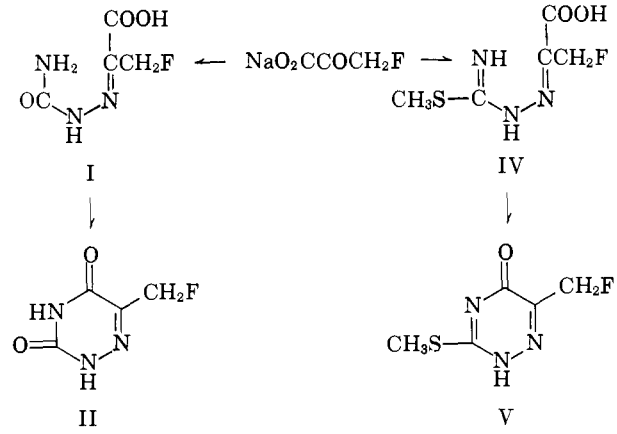
Received December 4, 1965

The current interest in 6-azauracil [*as*-triazine-3,5-(2H,4H)-dione] and its derivatives as potential anticancer agents[2,3] and in thiosemicarbazones as antiviral agents[4,5] has prompted the syntheses of the compounds described in this paper.

5-Fluoromethyl-6-azauracil [6-fluoromethyl-*as*-triazine-3,5(2H,4H)-dione] (II) was prepared by the ring closure of the known fluoropyruvic acid semicarbazone



(I).[6] The classical base-catalyzed ring closure of α-keto acid semicarbazones[7] to 3,5-dione-*as*-triazines failed, apparently due to the lability of the α-imino fluorine. Finally ring closure to II was accomplished by the method recently described by Heidelberger and Dipple.[8] Since fluoropyruvic acid 3-thiosemicarbazone (III) could not be cyclized to the *as*-triazine, fluoropyruvic acid 3-methylisothiosemicarbazone (IV) was used. Cyclization of IV to 3-(methylthio)-6-fluoromethyl-*as*-triazin-5(2H)-one (V) was effected in boiling water.[9] The infrared[10] and ultraviolet spectra[11] of this compound are similar to that of the known 3-(methylthio)-*as*-triazin-5(2H)-one, indicating the structure shown (V). The ease of cyclization of the semicarbazones I and IV indicates that they both have the *syn* configuration.

These compounds have been submitted to the National Institutes of Health, Cancer Chemotherapy National Screening Center, for anticancer and antiviral screening. Antiviral screening of I based on its ability to inhibit or prevent cytopathic effect (CPE) in tissue culture as found by the Viral Chemotherapy Section, Drug Evaluation Branch, CCNSC, National Cancer Institute, is given in Table I.

TABLE I

Virus system	Results[a]
Columbia SK	±
Vaccinia	+
Lymphocytes choriomeningitis	−

[a] ± = 25–50% CPE (cytopathic effect), + = 0–50%, and − = 50–100%. Negative results were obtained when I was tested in the *in vivo* Columbia SK virus system.

Experimental Section[12]

Fluoropyruvic Acid Semicarbazone (I).[6]—To a solution of 5.65 g (0.051 mole) of semicarbazide hydrochloride in 35 ml of water

(1) Supported largely by the Research Grant CA 08095-02 from the National Cancer Institute, Public Health Service.

(2) R. Lee Clark, Jr., "Cancer Chemotherapy," Charles C Thomas, Publisher, Springfield, Ill., 1961, pp 35–36.

(3) J. Skoda, *Progr. Nucleic Acid Res.*, **3**, 197 (1963).

(4) C. H. Stuart-Harris and L. Dickinson, "The Background to Chemotherapy of Virus Diseases," Charles C Thomas, Publisher, Springfield, Ill., 1964, pp 65–70.

(5) R. L. Thompson, *Advan. Chemotherapy*, **1**, 106 (1964).

(6) P. V. Nair and H. Basch, *J. Org. Chem.*, **23**, 137 (1958). The authors reported the melting point and analysis of I; however, since the preparation was not given, it is included in this paper.

(7) J. Gut, *Advan. Heterocyclic Chem.*, **1**, 204 (1963).

(8) C. Heidelberger and A. Dipple, Abstracts, 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965, p 13D.

(9) The facile cyclization of glyoxylic acid 3-methylisothiosemicarbazone has been reported by (a) A. R. Restivo and F. A. Dondzila, *J. Org. Chem.*, **27**, 2281 (1962); (b) P. K. Chang and T. L. V. Ulbricht, *J. Am. Chem. Soc.*, **80**, 976 (1958); and (c) E. Cattelain, *Bull. Soc. Chim. France*, **11**, 256 (1944).

(10) M. Horak and J. Gut, *Collection Czech. Chem. Commun.*, **28**, 3392 (1963).

(11) J. Jonas and J. Gut, *ibid.*, **27**, 1886 (1962).

(12) Melting points were determined using a Kofler hot stage. Ultraviolet absorption spectra were recorded by a Bausch and Lomb Spectronic 505 spectrophotometer. Infrared spectra were determined on a Perkin-Elmer Infracord spectrophotometer. Analyses were performed by Micro-Tech Laboratories, Skokie, Ill.